Association of longitudinal white matter degeneration and cerebrospinal fluid biomarkers of neurodegeneration, inflammation and Alzheimer's disease in late-middle-aged adults

Running Title: CSF biomarkers predict longitudinal white matter degeneration

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Abbreviations

DTI = Diffusion Tensor Imaging
WRAP = Wisconsin Registry for Alzheimer's Prevention
FA = fractional anisotropy
MD = mean diffusivity
APOE4 = apolipoprotein E gene (APOE4)
FH = (parental) family history
WM = white matter
GM = gray matter
FSL = FMRIB Software Library
ANTS = Advanced Normalization Tools
Cingulum-CC = cingulum adjacent to corpus callosum
Cingulum-HC = hippocampal cingulum

ABSTRACT (currently 215, maximum is 250)

Understanding longitudinal relationships between *in vivo* neural injury biomarkers and brain microstructure would provide insight into both healthy aging and Alzheimer's disease (AD) trajectories, as well as which subtypes of neurodegeneration affect white matter during middle age. We studied 151 participants with at least two diffusion tensor imaging (DTI) scans and at least one lumbar puncture from two large observational and longitudinally followed cohorts. Cerebrospinal fluid (CSF) was assayed for pathology generally specific to AD (Aβ42 and phosphorylated-tau), axonal degeneration (NFL), dendritic degeneration (neurogranin), and astroglial inflammation (YKL-40). For each CSF variable (baseline or two-year change), DTI metric (fractional anisotropy, FA, or mean diffusivity, MD), and region of interest combination we performed a linear mixed effects model to test our hypothesis that neural injury markers would be associated with indicators of worse white matter health overall (lower FA and higher MD) and/or progressively worsening white matter health over time (decreasing FA and increasing MD). In both baseline and longitudinal CSF models, all CSF markers except Aβ42 were associated with FA or MD in one or more region of interest. These findings support the hypothesis that CSF markers of neural injury and astroglial activation, perhaps more so than amyloid, are associated with DTI metrics of impaired WM integrity cross-sectionally and over time in a cognitively healthy, late-middle-aged sample of participants.

1 INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by several hallmark pathologies, including extracellular deposition of beta-amyloid (Aβ) plaques (Klunk et al. 2004; Nordberg 2004; Ikonomovic et al. 2008), and intracellular aggregation of hyper-phosphorylated tau (Alonso et al. 1996). An important question in AD research is the extent to which these characteristic pathologies – as well as other biomarkers of neural injury – reliably predict changes in brain microstructure before the onset of dementia. While gray matter (GM) volume and microstructure have been extensively studied in relation to AD, alterations in white matter (WM) are also associated with levels of amyloid and tau, and WM microstructure is highly implicated in the cognitive changes that occur throughout the disease progression (Scheltens et al. 1995; Takahashi et al. 2002; Bozzali et al. 2002; Huang et al. 2007; Zhuang et al. 2013). Therefore, further elucidating the relationships between biomarkers of AD, neurodegeneration, and white matter integrity is paramount for understanding how the brain changes in healthy aging and in AD, as well as for understanding which subtypes of neurodegeneration (e.g. axonal, dendritic) affect WM networks in middle age.

Magnetic resonance diffusion tensor imaging (DTI), is a widely used technique for assessing brain microstructure *in vivo*, and has proved invaluable for understanding WM alterations in AD. Among the data that DTI provides, mean diffusivity (MD) and fractional anisotropy (FA) are two of the most commonly studied. MD is sensitive to the average diffusion within a voxel of the brain, and is a surrogate marker for membrane integrity (Alexander et al. 2011). FA is sensitive to diffusion parallel to axons, and changes in FA are thought to reflect myelination or axonal degeneration (Alexander et al. 2011; Bendlin et al. 2010). One early study found that AD patients exhibited lower FA than controls in temporal lobe WM, the genu of the corpus callosum, and the cingulum bundle (Takahashi et al. 2002). Similarly, others have shown that FA is reduced in individuals with mild cognitive impairment (MCI), suggesting that loss of WM integrity occurs in early during the course of AD (Medina et al. 2006). With respect to MD, patients with AD exhibit higher values in the corpus callosum, and in WM in the frontal, temporal and parietal lobes (Bozzali et al. 2002). Similarly, one study found that MD is increased in the splenium and WM of the frontal and parietal lobes in patients with early AD suggesting that WM alterations are present before the onset of clinical dementia (Naggara et al. 2006).

In light of this evidence, which suggests that WM alterations may play a particularly important role in the progression from healthy cognition to dementia, it is important to predict and understand the earliest WM changes in healthy but at-risk populations. The use of cerebrospinal fluid (CSF) biomarkers of tau pathology, amyloid deposition, and neurodegeneration have been particularly valuable for understanding the evolution of early pathophysiology in AD (REF), and may be similarly useful in predicting WM microstructure. It is known that CSF biomarkers are altered before clinical cognitive symptoms, with levels of Aβ42 decreasing in tandem with brain

Aβ plaque deposition (REF), and levels of total and phosphorylated tau (p-tau) increasing as the time to diagnosis decreases (REF) (Jack et al. 2013). Similarly, other markers of axonal and dendritic neurodegeneration, such as neurofilament light protein (NFL) and neurogranin, are predictive of cognitive decline and are increased in AD patients compared to controls (Zetterberg et al. 2015; Janelidze et al. 2016). (I suggest these 2 refs instead of Janelidze)Finally, markers of inflammation or activated microglia and astrocytes, such as YKL-40, are thought to serve as proxies for neural injury (Craig-Schapiro et al. 2010; Antonell et al. 2014).

Several studies have used CSF biomarkers in tandem with DTI to assess how biomarkers of pathology predict brain microstructure in incipient or current AD. For instance, a 2011 study found that among 47 patients with subjective memory impairment or MCI, higher levels of CSF total-tau (t-tau) were predictive of lower FA in posterior cingulum fibers compared to healthy control individuals (Stenset et al. 2011). Similarly, another group found that more pathological levels of Aβ42 and t-tau are associated with reduced FA and increased MD in diffuse WM networks among a larger sample of 78 participants, including those with subjective cognitive impairment, MCI, and AD (Li et al. 2014).

Other work has looked for similar relationships between CSF biomarkers and brain microstructure in cognitively healthy older adults. For instance, it has been shown that CSF Aβ42 and tau are associated with reduced FA in the fornix, corpus callosum, and most robustly in the superior longitudinal fasciculus in 20 cognitively healthy older adults (Gold et al. 2014). Similarly, Bendlin et al. (2012) used levels of CSF biomarkers (Aβ42, tau, and NFL) as predictors for DTI metrics approximately 3.5 years later; the authors found that t-tau and t-tau/Aβ42 were associated with greater MD in WM of the temporal, parietal, and frontal lobes in 43 cognitively healthy older adults. Interestingly, and lending further credence to the idea that WM plays an important role in disease progression, these authors found no relationship between CSF biomarkers and GM volume. Moreover, another 2013 study found that 19 cognitively healthy individuals with low levels of CSF Aβ42 exhibited increased axial diffusivity in the corpus callosum, superior longitudinal fasciculus, fornix, and uncinate fasciculus, which the authors speculate may represent axonal atrophy (Molinuevo et al. 2014).

Importantly, longitudinal data examining the relationships between CSF biomarkers and brain microstructure are much more limited. It is particularly important to explore this gap in research because of the varying trajectories of WM microstructure in healthy aging and in AD (Ryan et al. 2013; Racine et al. 2014; Kim et al. 2015) – that is, cross-sectional DTI data may fail to capture the nuances of microstructural change over the disease course. In an attempt to understand this time course, Amlien et al. (2013) found that participants with MCI and high levels of CSF t-tau exhibited significant decreases in FA over a two-year time period compared to individuals without MCI or MCI-patients without pathological levels of CSF t-tau.

Considering these data, the present study sought to extend this line of research by examining the relationship between a more diverse panel of CSF biomarkers and brain microstructure longitudinally in a large sample of cognitively healthy older adults. In this way, this study aimed to determine the types of neurodegeneration most sensitive at predicting alterations in microstructure, including pathology generally specific to AD (Aβ42 and p-tau), axonal degeneration (NFL), synaptic degeneration (neurogranin), and inflammation (YKL-40). We postulated that greater CSF pathology at a baseline time point, and measured longitudinally, would be indicative of a more rapid progression toward pathological microstructure measured by DTI (e.g. decreasing FA and increasing MD).

2 MATERIAL AND METHODS

2.1 Participants

Participants were selected from one of two large observational and longitudinally followed cohorts at the University of Wisconsin-Madison: the Wisconsin Registry for Alzheimer's Prevention (WRAP; n=127, 84.1%) and the Wisconsin Alzheimer's Disease Research Center (WADRC; n=24, 15.9%) if they had undergone at least two DTI scans and at least one lumbar puncture. These cohorts have been described previously (Sager et al. 2005, Jonaitis et al. 2013, Koscik et al. 2014; A.M. Racine et al. 2016). All participants were cognitively normal at study entry. Cohorts did not differ by APOE4 carriage but did differ on sex and age at baseline MRI where ADRC had more females compared to WRAP (88% compared to 66%) and was younger on average at their baseline MRI (56.7±5.9 compared to 61.4±6.2) so both these variables were included as covariates in all models. To control for other possibly unmeasurable differences between cohorts we also included a dichotomous variable for cohort as a covariate in all analyses.

The University of Wisconsin Institutional Review Board approved all study procedures, each participant provided signed informed consent before participation, and all research was completed in accordance with the Helsinki Declaration.

2.1.1 DTI acquisition, processing, and signal extraction in regions of interest

DTI acquisition, image analysis, template creation, and spatial normalization have been described in detail previously (Racine et al. 2014; Kim et al. 2015) and were identical across cohorts. All participants in the WADRC dataset were imaged on one General Electric 3.0 Tesla Discovery MR750 (Waukesha, WI) MRI scanner, while all WRAP participants were imaged on a second, identical scanner. New methods included longitudinal registration of the longitudinally acquired DTI (n=152 with at least two scans, n=72 with three scans, n=19 with four scans, and n=3 with five scans).

Johns Hopkins ROIs (Wakana et al. 2004) were individually warped to the study's template space using ANTS. ROI maps were thresholded at 0.2 to reduce inclusion of gray matter voxels in the white matter masks using the FA map for each participant in normalized template space and then binarized. Mean FA and MD values were extracted from each ROI using fslstats. We chose to examine only FA and MD in these regions because they are the most commonly used metrics and to reduce the number of statistical tests performed.

White matter ROIs were selected based on a previous analysis examining the cross-sectional relationship between brain amyloid and white matter using amyloid imaging with PET-PiB and DTI scans acquired at a single time point (Racine et al. 2014). ROIs for the present study included a global white matter mask as well as the left and right cingulum divided into two parts: the cingulum-CC is defined as the portion of the cingulum that runs within the cingulate gyrus, traveling dorsally around the corpus callosum and then transitions to cingulum-HC as it progresses from the splenium of the corpus callosum along the ventral surface of the hippocampus, terminating at entorhinal cortex (Racine et al. 2014). Unlike the previous paper, we chose not to examine the fornix because despite functional interest in this region, CSF partial volume effects are extremely common in the fornix which make it difficult to study with DTI (Baron and Beaulieu 2015), especially in a longitudinal framework. Because the longitudinal nature of the present study prohibited whole-brain voxel-wise analyses, we also added a global white matter ROI. Figure 1 displays the chosen ROIs (forthcoming).

2.1.2 Cerebrospinal fluid

CSF was collected and assayed as described previously (Starks et al. 2015) and methods were identical across cohorts. We were primarily interested in CSF variables of neural injury, but included CSF measures of amyloid pathology (Aβ42 and Aβ42/Aβ40) for comparison. Neural injury was investigated by several different measures. Respectively, higher levels of neurofilament light protein (NFL), neurogranin, chitinase-3-like protein (YKL-40), and p-tau are indicative of greater axonal degeneration, dendritic degeneration, microglial activation and inflammation, and neurofibrillary tangles. In addition to examining p-tau alone, we also used a ratio of p-tau/Aβ42, which may be even more sensitive to AD and disease progression (Duits et al. 2014; Fagan et al. 2007; E. Racine et al. 2016). We also examined t-tau and t-tau/Aβ42 but these variables are known to be highly correlated with p-tau (Pearson's r=.894) and p-tau/Aβ42 (Pearsons's r=.942), respectively, in our sample. Results were highly consistent between the t-tau and p-tau measures so we only report results for p-tau.

CSF assays were performed in two batches, and due to methodological limitations, the values of a CSF analyte in one batch are not necessarily directly comparable to that same CSF analyte in a different batch. Therefore, we corrected for batch using simple linear regression (SLR) on a subset of CSF samples (n=96 from the entire WRAP and WADRC CSF datasets, regardless of

whether these participants had also undergone MRI) that were assayed in both batches. Associated predictions were made on what the CSF analysis values in batch 2 would be if they had been tested in batch 1. SLR was also used to test whether any predictions were necessary between the two batches using null hypothesis tests of an intercept of 0 and a slope of 1, both when batch 1 is a predictor and a response; if there was insufficient evidence to suggest that any of these hypotheses should be rejected, then an identify function is a reasonable prediction method (i.e., the batch 1 and batch 2 values were considered comparable without any prediction method between batches). If sufficient evidence existed to reject any of the null hypotheses, then predictions were made with SLR. Additionally, for some CSF variables, transforms were done to help correct residual violations that occurred in the non-transformed regression. In these instances, both the raw CSF values from batch 1 and the predicted CSF values from batch 2 were transformed accordingly. All analyses for CSF corrections were performed using R version 3.2.3 using the base "lm" function. Based on these methods, raw values were used for NFL and YKL-40 and predicted values are used for all other CSF variables; Aβ42 was transformed using the natural log.

2.2 Statistical analyses

For each CSF variable (baseline or change over two years), DTI metric (FA or MD), and ROI combination we performed a linear mixed effects (LME) model in Statistical Package for the Social Sciences (SPSS) 22 to test our hypotheses that CSF measures of neural injury would be associated with indicators of worse white matter health overall (lower FA and higher MD) and/or progressively worsening white matter health over time (decreasing FA and increasing MD). For each white matter ROI and DTI metric, we first ran unconditional growth models adjusting for random effects of intercept and time-associated slope to determine significant random effects, and then performed conditional models to tests our hypotheses. A significant β-coefficient on a CSF marker or change in CSF marker indicates that that predictor is associated with the DTI metric overall (intercept), whereas a significant β-coefficient on the predictor*time interactions indicate that the predictor is associated with change in the DTI metric over time (increasing/decreasing FA or MD). All models control for mean-centered age at baseline DTI scan, sex, and cohort. Results are evaluated at $p<.01$. Trends are reported for $p<.05$.

2.2.1 Baseline CSF and longitudinal white matter

Our first hypothesis was that CSF markers of neural injury measured at a single time point would be associated with DTI indices of poor white matter health and/or worsening white matter health over time. The conditional models included significant random effects as determined from the unconditional growth models, covariates, one of the CSF markers, time (interval between DTI scans), and the interaction between the CSF marker and time.

2.2.2 Change in CSF (ΔCSF) and longitudinal white matter

It's possible that increasing pathology as measured by change in CSF values (ΔCSF) could be more associated with longitudinal changes in white matter microstructure than the CSF level at a single time point. To test this hypothesis, we ran additional LME models in a smaller sample (n=91) who underwent a baseline lumbar puncture and a follow-up lumbar puncture approximately two years later (mean 2.16 years, SD 0.40, range 0.69-3.40). ΔCSF was calculated by subtracting the CSF level at visit 1 from the CSF level at visit 2. Therefore, positive values for ΔCSF indicate increasing pathology for all markers of neural injury, and negative numbers indicate increasing pathology for both amyloid markers. We performed separate unconditional growth models in this smaller sample and then conditional models which included significant random effects as determined from the unconditional growth models, covariates, baseline level of one of the CSF markers, ΔCSF for the same marker, time (interval between DTI scans), the interaction between the CSF marker and time (CSF*time), and the interaction between ΔCSF and time (ΔCSF*time).

3 RESULTS

3.1 Sample characteristics

Sample characteristics are summarized in Table 1.

Table 1. Sample characteristics

Sample Characteristics	**N**	**Mean/Frequency**	**SD**	**Range**
Age at baseline MRI	152	60.28	6.43	44.07 to 79.09
Sex (% female)		68.6%		
APOE4+		37.3%		
CSF Aβ42*	151	6.56	0.288	5.63 – 7.14
CSF Aβ42/Aβ40	150	0.096	0.018	0.042 – 0.138
CSF p-tau/Aβ42	149	0.061	0.027	0.0236 – 0.186
CSF p-tau	149	42.37	13.21	20.00 – 78.00
CSF NFL	150	625.25	243.15	222.00 – 2030.00
CSF Neurogranin	146	387.29	177.97	125.00 – 957.84
CSF YKL-40	151	145033.88	51388.87	49994.00 – 335820.87
ΔCSF Aβ42*	91	-0.039	0.155	-0.531 – 0.465
ΔCSF Aβ42/Aβ40	91	-0.001	0.010	-0.0264 – 0.023
ΔCSF p-tau/Aβ42	89	0.007	0.016	-0.037 – 0.060
ΔCSF p-tau	89	3.13	9.06	-33.00 – 30.44
ΔCSF NFL	89	80.510	207.76	-984.00 – 1070.00
ΔCSF Neurogranin	86	32.75	118.84	-417.93 – 657.13

ΔCSF YKL-40	91	11025.99	21155.65	-41812.00 – 99199.11
*CSF Aβ and ΔCSF Aβ42 were transformed by the natural log and so the transformed values are reported.				

3.2 Baseline CSF and longitudinal white matter

Higher p-tau/Aβ42 was significantly (p<.01) associated with higher MD in right (p=.005) and left (p=.007) cingulum-CC. Higher p-tau was associated with higher MD in right (p<.001) and left (p<.001) cingulum-CC. YKL-40 was associated with increasing MD over time in left cingulum-HC (p=.002).

There were no significant findings or trends for Aβ42 but several trends (p<.05) were observed for Aβ42/Aβ40 where lower Aβ42/Aβ40 was associated with lower FA in left cingulum-HC (p=.023) and increasing MD over time in the right (p=.049) and left (p=.014) cingulum-HC. Several other trends were also observed for the neural injury markers. Higher p-tau/Aβ42 was associated with increasing MD over time in left cingulum-HC (p=.013) and just missed the threshold for a trend for increasing MD over time in right cingulum-CC (p=.050). Higher p-tau was associated with lower FA in right cingulum-CC (p=.023) and increasing MD over time in left cingulum-HC (p=.027). Higher neurogranin was associated with increasing MD over time in left cingulum-HC (p=.024). Higher NFL was associated with lower FA in left cingulum-HC (p=.049).

A selection of these results are displayed in Figure 2.

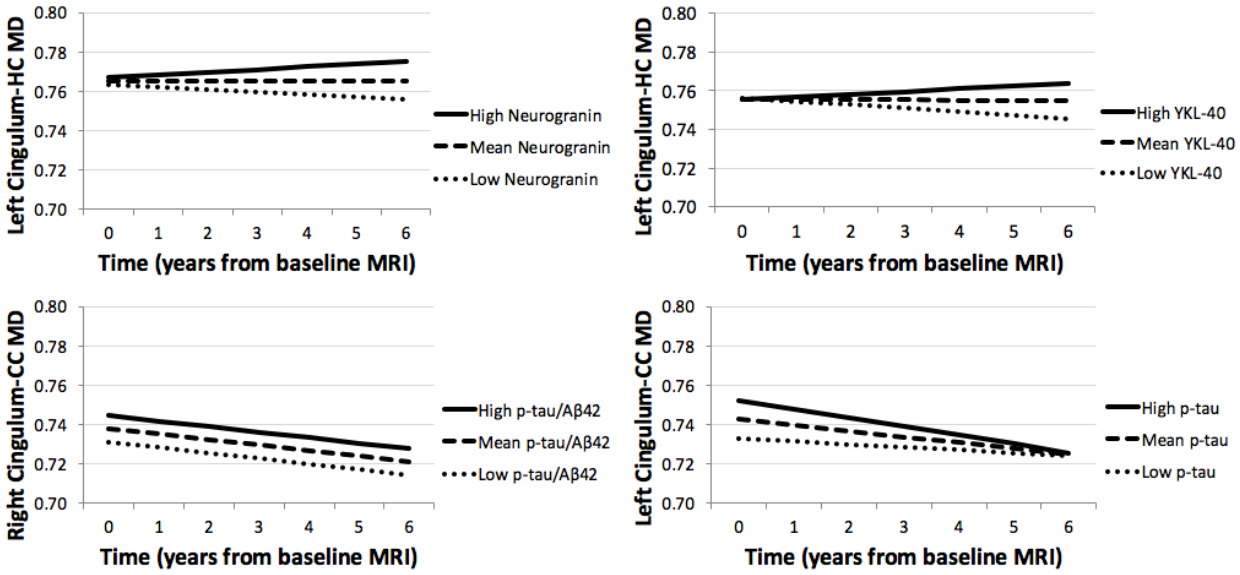

Figure 2. Associations between baseline CSF longitudinal white matter. Although the CSF variables were analyzed as continuous predictors, for visualization purposes they are displayed as high (1 SD above the mean, solid line), mean (dashed line), and low (1 SD below the mean, dotted line). Y-axis: Mean Diffusivity (MD) in the

specified ROI. X-axis: Time operationalized as interval from baseline MRI in years. Upper left: Neurogranin is associated with increasing MD over time; upper right: YKL-40 is associated with increasing MD over time; bottom left: p-tau/Aβ42 is associated with higher MD; bottom right: p-tau is associated with higher MD.

3.3 Change in CSF (ΔCSF) and longitudinal white matter

Higher p-tau/Aβ42 was significantly associated with decreasing FA over time in global white matter (p=.007). Higher neurogranin was associated with increasing MD over time in left cingulum-HC (p=.007). An increase in Δneurogranin was associated with higher MD in global WM (p<.001) and right (p<.001) and left (p<.001) cingulum-CC. Higher YKL-40 was associated with increasing MD over time in left cingulum-HC (p<.001).

Again, there were no significant findings or trends for Aβ42 but a trend was observed for Aβ42/Aβ40 where lower Aβ42/Aβ40 was associated with increasing MD over time in the right cingulum-HC (p=.038). Several other trends were also observed for the neural injury markers. Higher p-tau/Aβ42 was associated with lower FA in left cingulum-HC (p=.048) and increasing MD over time in right (p=.042) and left (p=.024) cingulum-HC. An increase in Δp-tau/Aβ42 was associated with increasing MD over time in left cingulum-CC (p=.041). An increase in Δneurogranin was associated with lower FA in global white matter (p=.010) and right (p=.034) and left (p=.035) cingulum-CC; and higher MD in right cingulum-HC (p=.022). Higher YKL-40 was associated with lower FA in left cingulum-HC (p=.035) and increasing MD over time in right cingulum-HC (p=.025). Unexpectedly, an increase in ΔNFL was associated with higher FA in right (p=.034) and left (p=.039) cingulum-HC.

A selection of these results are displayed in Figure 3.

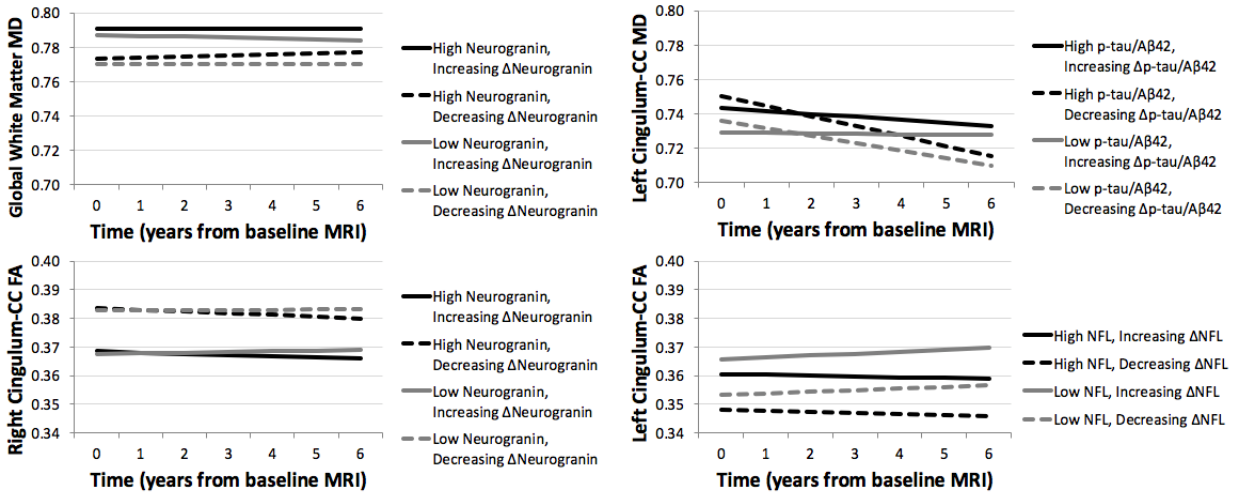

Figure 3. Associations between ΔCSF and longitudinal white matter. Although both baseline CSF and ΔCSF variables were analyzed as continuous predictors, for visualization purposes they are have been dichotomized as high/increasing (1 SD above the mean for CSF and ΔCSF, respectively) and low/decreasing (1 SD below the mean

for CSF and ΔCSF, respectively).Y-axis: Mean Diffusivity (MD) or Fractional Anisotropy (FA) in the specified ROI. X-axis: Time operationalized as interval from baseline MRI in years. Upper left: ΔNeurogranin is associated with MD; upper right: Δ p-tau/Aβ42 is associated with change in MD; bottom left: ΔNeurogranin is associated with FA; bottom right: ΔNFL is associated with FA.

4 DISCUSSION

Although CSF offers an indirect measure of pathological changes in the central nervous system, several CSF analytes have been identified which are relatively specific to different aspects of neurodegeneration in the brain. In the present study, we examined CSF markers of AD pathology (Aβ42, Aβ42/Aβ40, and p-tau) and neural injury through measures neurodegeneration in axons (NFL), and in dendrites (neurogranin) as well as inflammation/microglial or astrocytic activation (YKL-40). By associating cross-sectional and longitudinal measures of these analytes to longitudinally measured change in WM microstructure, we sought to better understand which subtypes of neurodegeneration affect WM networks during middle-age, and whether such markers can contribute predictive value to the more typically used AD markers of Aβ42 and p-tau.

In this study, we used linear mixed effects models with longitudinally measured DTI as the outcome and both baseline and longitudinal CSF measures of amyloid and neural injury as predictors to overcome some of the limitations of previous cross-sectional studies. Several CSF markers as well as change in CSF markers of both amyloid and neural injury were associated with altered WM microstructure consistent with an aging/AD-like signature (lower FA and higher MD) as well as temporally evolving degeneration of WM (decreasing FA and increasing MD) in regions vulnerable to AD.

In our baseline CSF models, all CSF markers except Aβ42 were associated with either higher FA or lower MD in several ROIs. The only ROI which showed measurable change over time in association with the CSF markers was the cingulum-HC. While several CSF markers (p-tau/Aβ42, Aβ42/Aβ40, p-tau, and neurogranin) showed trending relationships in this ROI, only YKL-40 was significantly associated with increasing MD over time in the cingulum-HC. These results suggest that markers of neural injury, perhaps more so than amyloid, are associated with WM deterioration, at least during middle age and possibly the presymptomatic phase of AD. Moreover, slight differences in findings between the neural injury markers suggest that certain types of degeneration differentially affect WM tracts, and the fact that YKL-40 was a particularly robust predictor for WM deterioration is in line with recent research implicating inflammation in the progression from healthy brain aging to pathology and dementia. Not only has it been shown that YKL-40 is generally higher among AD patients than age-matched healthy controls (Rosén et al. 2014; Wildsmith et al. 2014), but inflammation may be a particularly important phenomenon early in the AD progression. This is evidenced by a significant difference

in YKL-40 plasma and CSF concentrations in patients with early AD compared to healthy controls, but no difference in late stage AD patients compared to healthy controls (Choi et al. 2011; Antonell et al. 2014).

Although the sample size was smaller, the analyses examining longitudinal change in CSF markers provided additional insights into WM pathology compared to the cross-sectional snapshot. These analyses follow from previous studies demonstrating the utility of examining change in CSF and other aspects of disease progression. For instance, in a retrospective analysis, Stomrud et al. (2010) found that change (but not baseline) CSF levels of Aβ42 and p-tau predicted subsequent cognitive decline in a healthy older adult cohort. Additionally, Sutphen et al. (2015) found that increasing levels of t-tau, p-tau, YKL-40, and VILIP-1 (a marker of neuronal death) were associated with brain amyloid positivity measured by PET-PiB. This study further found that the majority of participants who progressed from a CDR of 0 to a CDR of 0.5 or 1 exhibited low Aβ42 and Aβ42/Aβ40 at baseline and follow-up as well as high total tau and t-tau/Aβ42. Finally, Amlien et al. (2013) assessed the predictive value of CSF biomarkers on WM integrity, showing that high levels of t-tau among MCI patients predicted significant decreases in FA over time.

The present study extends these findings by showing a relationship between longitudinal CSF biomarkers and longitudinal WM in a larger population of cognitively healthy participants. We found that longitudinal changes in CSF p-tau/Aβ2, neurogranin, and NFL over a 2-year time period were associated with baseline or longitudinal change in DTI metrics. Specifically, an increase in Δneurogranin was significantly associated with higher MD in global WM and cingulum-CC. At the trend level, an increase in Δneurogranin was also associated with lower FA and higher MD in several ROIs. Also at the trend level, an increase in Δp-tau/Aβ42 was associated with increasing MD over time in cingulum-CC, and an increase in ΔNFL was associated with higher FA in cingulum-HC. While this latter finding was unexpected, there are several plausible explanations for this result. As described before (Bendlin et al. 2012), higher NFL may predict higher FA if early axonal degeneration is present without simultaneous myelin loss, thereby retaining or increasing diffusion along the principal axis. Alternatively, higher NFL could predict higher FA if axonal degeneration results in a loss of crossing fibers specifically. Future studies should confirm or disprove these hypotheses.

On a more general level, this result is representative of the complexity of WM trajectories in healthy and pathological aging. A previous voxel-wise study from our group revealed cross-sectional differences in FA and MD measures across three levels of brain amyloid burden measured by PET-PiB (amyloid positive, indeterminate, and negative) where a general pattern of higher FA was observed in the Aβ positive and indeterminate groups compared to the Aβ negative group (Racine et al. 2014). In addition, using a new multi-resolution method for performing statistical analysis of connectivity networks/graphs derived from DTI tractography,

Kim et al. (2015) identified differences between healthy adults with and without a family history (FH) of AD. Similar to the previous study, they found that some WM connections had higher tract connectivity not only in the FH+ but also the FH- group. These studies suggest a complex relationship between AD risk and WM connectivity, and further necessitate longitudinal studies capable of mapping WM and biomarker trajectories in healthy aging and AD.

With the present results in mind and in the context of previous studies, it should be noted that our interpretations are limited by the fact that both CSF and DTI are indirect measures of biological processes and that our longitudinal measurements of both biomarkers are restricted to a relatively short interval when considered in the context of aging and age-related diseases, which unfold over decades. The smaller sample size in the longitudinal CSF analyses may also have restricted the power to detect additional relationships, and could partially explain the unexpected finding for ΔNFL and higher FA in the cingulum-HC. As such, these results warrant replication in future studies.

Despite these limitations, this study provides important insights into the evolving relationship between CSF markers of neural injury and WM microstructure in an aging middle-aged cohort enriched with risk factors for AD. Our results support the hypothesis that CSF indicators of neural injury, which encompass markers of general neuronal degeneration, axonal degeneration, dendritic degeneration, and inflammation, perhaps more so than amyloid, are associated with DTI metrics of impaired WM health. A critical next step will be to connect these longitudinal biomarker changes to cognitive decline and diagnosed disease. Follow-up studies of these participants are ongoing, and the results from the current study will be especially useful in the context of future longitudinal assessments.

5 CONFLICT OF INTEREST

KB and HZ are co-founders of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg. KB has served as a consultant or at advisory boards for IBL International, Roche Diagnostics, Eli Lilly, Fujirebio Europe, and Novartis. The other authors declare that they have no conflict of interest.

6 ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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